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Patterning with Diffusion Barriers

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Notch signaling instructs equivalent cells to form precise differentiation patterns. In this issue of *Developmental Cell*, Cinquin et al. (2015) characterize diffusion barriers that enhance Notch patterning within the *Caenorhabditis elegans* gonad.

Noise can be annoying. Your cells think so, too. Cell signaling molecules called morphogens have fascinated developmental biologists for a long time (Wolpert, 1996). During development, cells interpret morphogen gradients into cell fate transitions that occur at discrete boundaries (Ashe and Briscoe, 2006) (Figure 1A). A key question is how cells convert a signaling gradient into a precise pattern. Central to this question is the concept of noise (Lander et al., 2009). Morphogen levels vary in time and space, yet cells interpret the gradient correctly. Noise is most problematic when morphogen concentration is low or, metaphorically, when your voice is hoarse. In this issue of *Developmental Cell*, Cinquin et al. (2015) discover a mechanism that buffers against noisy Notch signaling in the *C. elegans* gonad. At the core of this mechanism is a tissue remodeling process for slowing diffusion.

The *C. elegans* hermaphrodite gonad is a model for studying stem cells, meiosis, fertilization, and myriad other fundamental processes (Hubbard and Greenstein, 2005). The germline develops in a syncytium (Figure 1B). Mitotically proliferating germ cells (forming a mitotic zone) and their meiotically progressing descendants share a common cytoplasm called the rachis, which is spanned by internal germ cell bridges. At the gonad tip lies the distal tip cell (DTC), a somatic cell with finger-like projections covering the germline stem cells (Hansen and Schedl, 2013; Kimble and Crittenden, 2005). The Notch ligand LAG-2 is expressed in the DTC, whereas the Notch receptor GLP-1, CSL DNA-binding protein LAG-1, and transcriptional activator LAG-3 are expressed in the germline (Byrd and Kimble, 2009; Greenwald and Kovall, 2013). Notch signaling promotes germline stem cell self-renewal. In the absence of Notch, germ cells stop proliferating and enter meiosis.

LAG-2 in the DTC is thought to act as a morphogen for patterning the distal germline. Germ cell fate transitions, such as entry into the transition zone, occur as germ cells are displaced proximally (Figure 1B). Transitions can be observed using a differentiation marker called GLD-1 or nuclear morphology. Cinquin et al. (2015) started with a simple experiment: they injected diffusible fluorescent dyes into the germline syncytium. What they found were steps in dye concentration that mapped to the internal germ cell bridges spanning the rachis. The bridges form 1-μm diameter constrictions, creating a diffusion barrier. The authors then used single-point fluorescence correlation spectroscopy to measure fluorescent dextran concentrations. Fitting them to Fick's second law of diffusion, which describes the rate at which concentration changes at a given point over time, yielded a diffusion coefficient of $\sim$20 $\mu m^2 \cdot s^{-1}$, roughly similar to that of GFP within cells. Next the authors built a mathematical model to test whether rachis constriction is sufficient to generate concentration steps. The conclusion is that modeling was consistent with experimental observations. Steps in concentration persisted for about an hour until a steady state was reached.

The authors suspected that the diffusion barriers might mark changes in germ cell differentiation state. Indeed, the rachis constrictions coincided with boundaries in GLD-1::GFP reporter gene expression and nuclear morphology. Moreover, mitotic zone size correlated well with diffusion barrier position. When oil droplets were injected into the syncytium to create artificial diffusion barriers, these barriers reduced mitotic zone size. The authors previously showed that inactivation of *emb-30*, an anaphase-promoting complex subunit, causes two germ cell populations to form: a distal pool that failed to enter meiosis and a proximal pool that succeeded in doing so. Cinquin et al. (2015) found a diffusion barrier at the boundary between these pools.

What about the relationship between Notch and the diffusion barriers? Downregulating Notch signaling using a temperature-sensitive mutant caused germ cells to differentiate, and differentiation was preceded by loss of diffusion barriers and GLD-1 expression steps. Next, the authors attempted another clever experiment. They injected oil droplets into Notch mutant gonads, essentially re-introducing the diffusion barrier. In the presence of the introduced barrier, differentiation of the most distal germ cells was delayed. The results support the notion that diffusible factors within the distal germline syncytium control differentiation. Notch signaling appears to create diffusion barriers within the rachis, slowing flux of differentiation factors and forming steps in local concentration.

Cinquin et al. (2015) measured nuclear levels of GLP-1::GFP, a readout of Notch signaling activity. They found extensive variability in nuclear Notch concentration within and across gonad samples, much more so than in mitotic zone size. These data suggested that diffusion barriers provide a noise buffer for controlling the mitotic zone. Mathematical modeling was used to further test this idea. The model that best fit experimental data included diffusion barrier positions regulated by Notch and feedback. This model

correctly reproduced the GLD-1 steps, diffusion barrier locations, and positive correlation between barrier position and mitotic zone size.

To experimentally test their mathematical model, the authors turned to the less-popular male gonad. In males, the rachis does not contain diffusion barriers, yet does contain a mitotic zone. They found that mitotic zone size is less robustly maintained in males than in hermaphrodites as temperature increases. Now the authors went back to the model. When diffusion barriers were removed from the syncytium, patterning robustness against changes in Notch signaling was reduced. With barriers, the system maintained robustness when Notch was perturbed spatially in a manner that left total Notch signaling unchanged. Furthermore, adding a negative feedback step (supported by experimental evidence) yielded a model in which mitotic zone size was sensitive to total Notch activity but insensitive to variability in Notch spatial distribution. The take-home message is that diffusion barriers buffer against natural signaling variation, in essence translating a signaling gradient into discrete germ cell fate decisions.

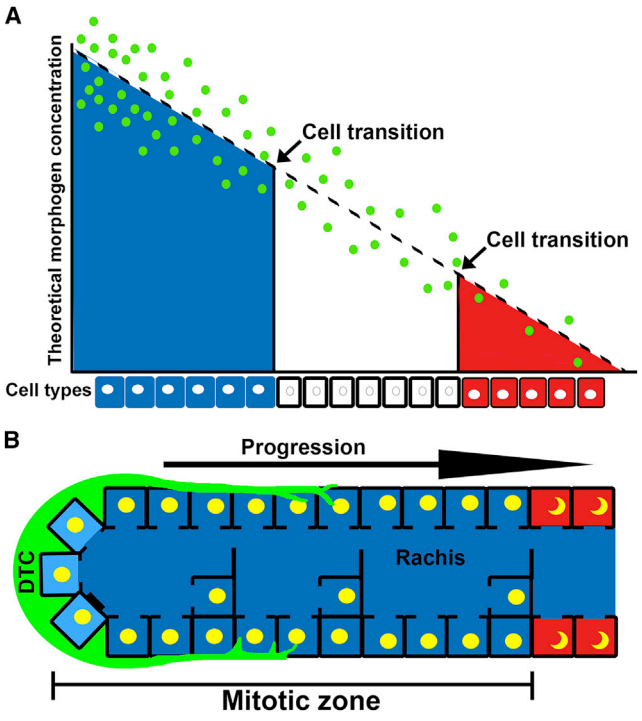

Figure 1. Morphogen Patterning and the *C. elegans* Distal Gonad

(A) Classic French flag morphogen signaling model. Equivalent cells determine their position by reading a morphogen gradient, which originates from a source (left). Cells sense whether they are above or below a concentration threshold, triggering a cell transition that includes gene expression changes. Transitions form discrete boundaries of differentiated cells (below). In reality, morphogen concentration (illustrated by green dots) and downstream signaling activity vary due to molecular noise. How cells deal with noise to generate robust differentiation patterns is the subject of the paper by Cinquin et al. (2015). (B) *C. elegans* distal gonad. Germline stem cells (light blue) and their descendants (dark blue and red) develop in a syncytium that contains cell bridges within the rachis. Germ cells progress proximally through the mitotic zone before entering the transition zone (marked by red cells). The mitotic zone contains cells in the mitotic cell cycle and meiotic S phase. The transition zone is characterized by distinct crescent-shaped nuclei (yellow) characteristic of meiotic prophase. The GLD-1 translational repressor acts downstream of Notch signaling to promote meiotic entry. Steps in GLD-1 expression are seen in the mitotic zone, suggesting that they mark cell fate transitions (not shown).

In theory, the semi-permeable diffusion barriers Cinquin et al. (2015) describe could be important for intracellular and extracellular morphogens. There are many examples of syncytial organization, including fly embryos, the vertebrate lens, and developing mammalian germ cells. It is also interesting to think about how tissue structure might influence diffusion through extracellular spaces. Like any provocative manuscript, more questions emerged at the end than existed at the beginning. For instance, what are the diffusible differentiation factors within the rachis? How does Notch generate the diffusion barriers? And finally, do these barriers affect other signaling pathways? Diffusion barriers might be the cell's answer to family living: partition rooms so that communication can be better confined to appropriate audiences, without interferences from noisy neighbors.

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